In Chapter 1 an introduction on herpesviruses and type-specific serology is given Herpes simplex virus (HSV) is one of the best-studied herpesviruses. Despite this many of its properties are still unknown. Herpesviruses are present in a large part of the population and it is common that infected individuals transmit the virus withou knowning that they are infected. After a primary infection herpesviruses become latent and latency lasts lifelong. During latency, the virus can be reactivated from its latent state and give recurrences or reactivations. The recurrences can be symptomatic or asymptomatic and during both episodes virus shedding can occur. HSV-1 used to be a pathogen of orolabial herpesvirus infections and HSV-2 used to be a pathogen of genital herpesvirus infections. Recently HSV-1 was found to be associated with genital infections and HSV-2 was found in the oral region. With serological screening HSV-1 and HSV-2-infected individuals can be detected by the presence of HSV type-specific antibodies. Virus detection as diagnostic tool for identification of the infected individuals is only possible during the acute phase of an active infection. Detection of type-specific antibodies has been hampered in the past by the extensive serological cross-reactivity between HSV-1 and HSV-2. At present, commercial assays are available (Chapter 1), based on the type-specific antigens gG-1 and gG-2 and they can be used for the detection of HSV-1 and HSV-2 type-specific antibodies.

The determination of the seroprevalence of HSV-2 and HSV-1 in defined populations in Groningen was the main aim of the study described in this thesis. Commercial assays are expensive because of the high costs of antigen preparation. Therefore their use in large-scale surveys is limited. As a solution to the cost aspects, we developed an "in house" assay by cloning of the type-specific glycoproteins G-1 and G-2 (Chapter 2). We used the baculovirus expression system, which is easy and safe to work with and convenient to produce relatively large amounts of proteins. We started cloning of the complete sequence of gG-2 and a truncated version of gG-2, resulting in recombinant baculoviruses that expressed very little gG-2. In addition these recombinant viruses were not stable. Therefore, we decided to clone a smaller fragment of the gG-2 sequence, coding for amino acid residues 281-594. The latter fragment contains most of the known epitopes of gG-2 and the proposed cleavage sites that allow processing. In order to improve the expression, the gG-2 signal sequence was replaced by the honeybee mellitin signal sequence. Six histidines were added to the C-terminus to allow purification by affinity chromatography. In addition, gG-1 (residues 26-189, gG-$1_{t26\text{-}189His}$), and gD-1 (residues 1-313, gD-$1_{1\text{-}313}$) were cloned in the baculovirus expression system. The production of recombinant gG-$2_{281\text{-}594His}$ and gG-$1_{t26\text{-}189His}$ was confirmed by Western blot using type-specific monoclonal antibodies (MAbs, AP1 and LP10, respectively) and specific human sera. The molecular masses of the major products of gG-$1_{t26\text{-}189His}$ and the fragment of gG-$2_{281\text{-}594His}$ were 36-39 kDa and 64-72 kDa, respectively. Human sera positive for HSV-1 reacted with gG-$1_{t26\text{-}189His}$, sera positive for HSV-2 reacted with the gG-$2_{281\text{-}594His}$ fragment, and sera positive for both types reacted with gG-$1_{t26\text{-}189His}$ and gG-$2_{281\text{-}594His}$ in Western blot. The human sera recognized polypeptides of gG-$2_{281\text{-}594His}$ with molecular masses of 57-67 and 120-150 kDa and additional faint bands of 21, 29, and 45 kDa. The recombinant gG-$1_{t26\text{-}189His}$ and the recombinant gG-$2_{281\text{-}594His}$ fragment were used as type-specific antigens for the detection of HSV-1 and HSV-2 specific antibody responses in human sera, respectively. As type-common antigens, an extract

of HSV-1 infected Vero cells and recombinant gD-$1_{1\text{-}313}$ were used. An ELISA to detect type-specific antibodies was developed and the sensitivity and specificity were evaluated by comparison with commercial tests using sera obtained from different sources. The sensitivity and specificity were 91.5 % and 95.5 %, respectively, compared to the first version of the Gull assay as it was distributed by GULL Laboratories, Meridian Bioscience, Inc. Cincinnati, USA. The gG-$2_{281\text{-}594His}$ fragment could be obtained in relatively large quantities at low cost.

After we had developed this ELISA for the detection of HSV-1 and HSV-2 type-specific antibodies, it became possible to perform follow-up studies and to investigate whether antibody titers against different antigens derived from HSV change over time (Chapter 3). To this end, we analyzed the follow-up sera of HSV isolation-positive patients who attended the outpatient STD clinic of the Municipal Health Service (GG/GD), Amsterdam. We used 4 antigens *i.e.*, the extract of HSV-1 infected cells and baculovirus-expressed gD-1 (gD-$1_{1\text{-}313}$) as type-common antigens, and baculovirus-expressed gG-1 (gG-$1_{t26\text{-}189His}$) and a fragment of gG-2 (gG-$2_{281\text{-}594His}$) as type-specific antigens for the determination of antibodies. Among 145 genital HSV culture-proven patients, HSV-1 was isolated from 30 patients (20.7%). Twenty of them had only antibodies against HSV-1. The number of women (30%) with genital HSV-1 infections was significantly higher than the number of men (12%) with genital HSV-1 infections (p= 0.008). Follow-up sera were available of 103 out of 145 patients. Based on the virus isolation together with serological data of the follow-up sera, the patients were diagnosed as having primary infections, nonprimary initial infections, and recurrences at the time of visit to the clinic. Fifteen patients had primary infections, 4 patients had nonprimary initial infections, and 84 patients had recurrences. Antibodies against HSV and/or gD appeared earlier or at the same time as antibodies against gG-1 and gG-2. Except for in 5 patients (4 had a primary and 1 a recurrent infection) whose antibodies against HSV and gG were present earlier than the antibodies against gD. The mean antibody titer to HSV in HSV-1 isolation positive patients was 2484 and 6140 in HSV-2 isolation positive patients. The mean gD titers were 1817 in HSV-1 isolation positive patients and 5030 in HSV-2 isolation positive patients. The mean gG-1 titer was 628 in HSV-1 isolation positive patients and 641 in HSV-2 isolation positive patients. The mean gG-2 titers were 411 in HSV-1 isolation positive patients and 694 in HSV-2 isolation positive patients. Each mean titers were higher in HSV-2 isolation positive patients.

Among 15 patients with primary infections, all developed antibody titers against HSV, but 7 and 4 patients did not develop antibodies against gD and gG, respectively. The nonprimary infected patients had antibodies against HSV, gD, gG-1 and gG-2. Two of them lost type-specific antibodies against gG. One of the two had changes in HSV, gD and gG-1 antibody titers as well. Of the 84 patients with recurrent infections 47 had stable antibody titers against HSV, gD and gG. Twelve of the patients with recurrent infections did not develop gG type-specific antibodies. Changing antibody titers were found in 13 patients, 5 of which were among the twelve patients that did not develop gG type-specific antibodies at the time of virus isolation. Four patients with recurrent infections lost type-specific antibodies, two of them were among the 13 patients with changes in antibody titers. It was clearly demonstrated that in HSV-1 and HSV-2 isolation positive genital herpes patients changes in antibody titers and sometimes loss of type-specific antibodies may occur over time.

Next, the HSV-1 and HSV-2 seroprevalence in two defined populations, the STD clinic attenders of the Department of Dermatology in Groningen, and a population of women with dyskaryotic smears at risk for cervical cancer, attending the Gynaecology Department, also from the University Hospital in Groningen, were determined.

For determination of the HSV-1 and HSV-2 prevalence of STD clinic attenders, samples were collected from the patients who were admitted to the hospital and requested screening for STDs, mainly for syphilis and/or HIV infections (Chapter 4). A total of 493 serum samples was investigated. The samples were collected from October 1996 to May 1998 at the STD clinic of the Department of Dermatology of the University Hospital Groningen, The Netherlands. The overall seroprevalence of HSV-1 and HSV-2 in the STD clinic attenders was 70.0% and 17.2%, respectively. The seroprevalence of HSV-1 was higher in males and the HSV-2 seroprevalence was higher in females. Both HSV-1 and HSV-2 seroprevalence was significantly higher in the population of non-Dutch origin. The seroprevalence of HSV-2 was significantly higher in the populations who had current other STDs, *i.e.* HIV positive and syphilis positive patients. The seroprevalence of HSV-1 and HSV-2 was significantly higher in the population with a history of STD. Homosexual and bisexual orientation was associated with HSV-1 and HSV-2 seropositivity. The acquisition of HSV-2 antibodies occurred at younger age in females than in males. The STD clinic has a policy that testing is performed anonymously on patients' requests. Out of 493 patients 120 (24.3%) requested anonymous testing. Among those 120 patients, the HSV-1 and HSV-2 seroprevalence was significantly higher in females than that in males (80% versus 56.4%, 27.7% versus 5.5%, respectively). The seroprevalence of HSV-2 of the STD clinic attenders in Groningen was slightly lower but similar to the HSV-2 seroprevalence in other STD groups, *i.e.* Rotterdam, The Netherlands.

The second population of which the seroprevalence of HSV-1 and HSV-2 was determined was a group of 296 women with dyskaryotic smears at risk for cervical cancer (Chapter 5). Serum samples from 296 women with dyskaryotic smears were collected and screened for the presence of HSV-1 and HSV-2 antibodies by our type-specific "in house" ELISA. The recruited women responded to a questionnaire with regard to age of first pregnancy, age of first sexual intercourse, smoking habits, lifetime number of sexual partners, oral contraception use, history of sexually transmitted diseases (STDs), fever blisters, and (peri-) vulval warts to analyze risk factors associated with HSV seropositivity. The seroprevalence of HSV-1 and HSV-2 infections in this population was 89.9% and 38.5%, respectively. HSV-1 seropositivity was relatively high when compared with female STD clinic attenders, and was associated with seropositivity for *Chlamydia trachomatis* (*C. trachomatis*). Several studies have indicated an increasing incidence of genital herpes caused by HSV-1. This has been attributed to "changes in sexual practice" together with a decrease of HSV-1 infections in childhood. In our study, the HSV-1 seropositivity in the women with dyskaryotic smears was significantly associated with the presence of antibodies against *C. trachomatis*, which linked HSV-1 seropositivity to the occurrence of one of the most frequent STDs. When more stringent statistics (method of Bonferroni) were applied, the association of HSV-1 seropositivity and the presence of antibodies against *C. trachomatis* did not remain. The seroprevalence of HSV-2 in women with dyskaryotic smears is relatively high as well for the geographic area of

Groningen, where in Dutch female STD attenders a relatively low HSV-2 seroprevalence of 12.0% was found. The high HSV-2 prevalence in women with dyskaryotic smears cannot be explained by any of the known risk factors for the presence of HSV-2 antibodies.

Understanding of the HSV-1 and HSV-2 seroprevalence in the above-mentioned populations together with the already known data of the HSV-1 and HSV-2 seroprevalence in The Netherlands can provide information to decide whether effective prevention programmes are required for genital herpes in particular and for STD populations in general.